No Anisotropic Codon Cube Beats G-A-C-U: Exact Axis-Separable Proof and Exhaustive Search of 13,824 Genetic-Code Topologies

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The standard genetic code can be embedded in a 4 × 4 × 4 lattice whose axes represent the first, second, and third codon positions. Previous work showed that the isotropic nucleotide order G-A-C-U, together with its mirror U-C-A-G, uniquely maximizes synonymous nearest-neighbor connections at forty-four. This study asks whether a higher value can be obtained when the three codon axes are allowed to use independent nucleotide orders. The unrestricted search contains 24^3 = 13,824 anisotropic axis-order triples. Exhaustive enumeration shows that no case exceeds forty-four synonymous self-loops. The result is also explained analytically by exact axis separability: the total loop count is the sum of independent contributions from the first, second, and third codon axes. Their separate maxima are 4, 1, and 39, fixing the global bound at 44. Although 192 oriented axis triples attain this value, they reduce to 24 undirected solutions after reversal symmetry is removed. G-A-C-U therefore remains globally optimal in value, while isotropic uniqueness expands into a structured anisotropic family of ties.

Keywords: genetic code, anisotropic codon cube, G-A-C-U nucleotide order, synonymous self-loops, codon topology, axis-separable graph theory, 13,824 codon orderings, forty-four-loop bound, exhaustive enumeration, codon-position asymmetry, amino-acid multigraph, reversal symmetry, nearest-neighbor codons, genetic-code geometry, topological compactness, computational biology.

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Outline

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9.5 Complete loop-count distribution

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Caption: Distribution of synonymous self-loop counts across all 13,824 anisotropic codon cubes. No independently ordered axis triple exceeds forty-four loops.

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Identify G-A-C-U and U-C-A-G as the only isotropic maximizing cases.

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Place a table or diagram here showing the Cartesian product of the three optimal axis-order sets.

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Explain why G-A-C-U remains globally optimal even though anisotropic alternatives tie its value.

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12.5 Complete classification

Summarize the 192 oriented and 24 reversal-reduced maxima.

12.6 Final conclusion

Conclude that the unrestricted forty-four-loop bound is both analytically proven and exhaustively verified.

13. References

1. From the Isotropic Maximum to the Anisotropic Problem

1.1 The Ordered Codon Cube

The standard genetic code contains sixty-four codons formed from the four RNA nucleotides A, G, C, and U. Each codon is an ordered triplet with distinct first, second, and third positions. The complete codon set therefore contains

$4^3 = 64$

possible nucleotide words.

These codons can be represented as the vertices of a $4 \times 4 \times 4$ lattice. The first spatial axis corresponds to the first codon position, the second axis corresponds to the second position, and the third axis corresponds to the third position. Each axis contains four nucleotide states.

A linear order must be assigned to the nucleotides on each axis before nearest-neighbor relationships can be defined. For example, the order

G-A-C-U

contains the consecutive nucleotide pairs

G-A, A-C, and C-U.

Two codons are nearest neighbors in the ordered cube when they differ at exactly one codon position and the two nucleotides at that position are consecutive in the selected axis order. The other two codon positions must remain unchanged.

Each axis contains three adjacent nucleotide intervals. For every interval, the other two codon positions can each assume four values. The number of edges parallel to one axis is therefore

$(4 - 1) \times 4^2 = 48.$

The complete cube contains three axes, so the total number of undirected nearest-neighbor edges is

$3 \times 48 = 144.$

Every codon vertex is labeled by the amino acid it encodes. Translation stops are labeled X. If two neighboring codons encode the same amino acid or stop signal, their connection is synonymous.

The sixty-four codon vertices can also be collapsed according to translated identity. After this collapse, the graph contains twenty amino-acid vertices and one stop vertex. A synonymous codon edge then becomes a self-loop at the corresponding translated vertex.

The number of self-loops depends on the order assigned to the nucleotides. Changing an axis order changes which one-nucleotide substitutions are represented as nearest-neighbor lattice edges, even though the standard genetic-code assignments remain unchanged.

The ordered codon cube therefore converts synonymous degeneracy into a measurable graph property.

1.2 The Isotropic G-A-C-U Result

An isotropic codon cube uses the same nucleotide order on all three axes. If the common order is denoted by π, then the first-, second-, and third-position axes all use π.

Because four nucleotides can be arranged in

$4! = 24$

linear orders, there are twenty-four isotropic codon cubes.

For a selected order π, let $E\pi$ denote the set of 144 nearest-neighbor edges. Define $I\phi(e)$ to equal 1 when the two codons joined by edge e have the same translated label and 0 otherwise.

The synonymous self-loop count is then

$L(\pi) = \Sigma e \in E\pi \, I\phi(e)$.

Previous exhaustive enumeration of all twenty-four isotropic orders established that the largest loop count is

$\max L(\pi) = 44$.

Only two oriented orders attain this value:

G-A-C-U

and

U-C-A-G.

These two orders are complete reversals of one another. Reversing a linear path preserves its undirected adjacent nucleotide pairs. G-A-C-U contains the pairs G-A, A-C, and C-U, while U-C-A-G contains the same pairs in reverse order.

The two maximizing orders therefore represent one undirected topology viewed in opposite orientations.

The earlier result may be stated precisely:

G-A-C-U is the unique isotropic maximum up to reversal.

For the G-A-C-U cube, the forty-four synonymous edges are distributed very unevenly among the three codon axes. The contributions are

$(L_1, L_2, L_3) = (4, 1, 39)$.

Thus, four synonymous edges occur along the first-position axis, one occurs along the second-position axis, and thirty-nine occur along the third-position axis.

This extreme asymmetry reflects the structure of the standard genetic code. Third-position substitutions frequently preserve amino-acid identity, whereas second-position substitutions almost always alter it. First-position substitutions produce a small number of synonymous bridges between separated codon blocks.

The isotropic result established that G-A-C-U simultaneously accommodates all three positional structures better than any other single nucleotide order.

1.3 Why Anisotropy Matters

The isotropic condition imposes one common nucleotide geometry on all three codon positions. This is mathematically elegant, but it is biologically restrictive because the three positions have very different patterns of degeneracy and amino-acid assignment.

The third position is dominated by synonymous variation. Many twofold-degenerate codon families use the pairs G-A or C-U at the third position. Complete fourfold-degenerate families occupy all four third-position nucleotide states.

The first position contains a much smaller number of synonymous connections. These occur mainly where sixfold-degenerate amino acids are divided between separate codon blocks.

The second position is different again. It strongly organizes amino-acid physicochemical properties, but it contributes almost no synonymous nearest-neighbor structure.

There is therefore no initial reason to assume that the nucleotide order maximizing first-position loops must also maximize second- or third-position loops.

An anisotropic cube removes this restriction by assigning independent orders to the three axes:

π_1 for codon position one,

π_2 for codon position two,

and

π_3 for codon position three.

The first axis can then use an order favoring first-position synonymous bridges, while the third axis can use an order favoring third-position degeneracy. The second axis can be optimized independently for its small remaining synonymous contribution.

This creates the possibility that a mixed combination of axis orders might outperform every isotropic cube.

The anisotropic problem is therefore not a minor extension. It tests whether the forty-four-loop result depends on forcing one order to perform three different positional tasks simultaneously.

1.4 The Unrestricted Question

Each codon axis admits twenty-four oriented nucleotide orders. Allowing the three axes to vary independently produces

$24 \times 24 \times 24 = 24^3$

axis-order triples.

Therefore, the complete anisotropic search space contains

$24^3 = 13{,}824$

ordered cubes.

The twenty-four isotropic cases are those satisfying

$\pi_1 = \pi_2 = \pi_3$.

All remaining cases are anisotropic because at least two axis orders differ.

The central question of this paper is:

Can any independently ordered codon cube produce more than forty-four synonymous nearest-neighbor edges?

The most direct approach is exhaustive enumeration. Every one of the 13,824 axis-order triples can be constructed, its 144 edges generated, and its synonymous edges counted.

However, exhaustive computation alone does not explain the result. A stronger analysis asks whether the three axis contributions can be separated mathematically.

If the total loop count decomposes into independent first-, second-, and third-axis terms, then the unrestricted maximum can be found by maximizing each axis separately.

The anisotropic problem therefore has two complementary parts:

1. an analytical proof based on axis separability;
2. an exhaustive computational verification across all 13,824 cases.

Agreement between these approaches provides both explanation and independent confirmation.

1.5 Principal Result

The exhaustive search shows that no anisotropic ordering exceeds forty-four synonymous self-loops.

The unrestricted maximum is

$L_{max} = 44$.

The analytical reason is that the total loop count separates exactly by codon axis:

$L(\pi_1, \pi_2, \pi_3) = L_1(\pi_1) + L_2(\pi_2) + L_3(\pi_3)$.

The maximum first-axis contribution is

$\max L_1 = 4$.

The maximum second-axis contribution is

$\max L_2 = 1$.

The maximum third-axis contribution is

$\max L_3 = 39$.

The unrestricted maximum must therefore be

$L_{max} = 4 + 1 + 39 = 44$.

The G-A-C-U isotropic cube already attains all three axiswise maxima simultaneously:

$(L_1, L_2, L_3) = (4, 1, 39)$.

Allowing different orders on the three axes cannot increase any of these contributions beyond its individual maximum. Anisotropy therefore cannot improve the total.

The expanded search does change the uniqueness result. Across all anisotropic cases,

0 cases exceed 44,

while

192 oriented axis-order triples attain 44.

These 192 maxima arise from combining four optimal first-axis orders, twelve optimal second-axis orders, and four optimal third-axis orders:

4 × 12 × 4 = 192.

Reversal of an axis preserves its undirected adjacency path. After reversal-equivalent orders are identified, the 192 oriented maxima reduce to

24 distinct undirected axis-order triples.

The principal conclusion is therefore:

Independent axis ordering creates additional maximizing configurations but does not increase the forty-four-loop bound.

G-A-C-U remains globally optimal in value. Its uniqueness is specific to the isotropic search, while the unrestricted anisotropic search reveals a larger, exactly classifiable family of ties.

2. Mathematical Construction of an Anisotropic Codon Cube

2.1 Complete Codon Space

The RNA nucleotide alphabet contains four symbols:

$\mathcal{A}$ = {A, G, C, U}.

A codon is an ordered nucleotide triplet,

$c = (c_1, c_2, c_3)$,

where c_1 is the nucleotide in the first codon position, c_2 is the nucleotide in the second position, and c_3 is the nucleotide in the third position.

The complete codon space is the Cartesian product

$\mathcal{C} = \mathcal{A} \times \mathcal{A} \times \mathcal{A}$.

Equivalently,

$\mathcal{C} = \mathcal{A}^3$.

Because each of the three positions can contain any of four nucleotides, the number of codons is

$|\mathcal{C}| = 4^3 = 64$.

Every possible RNA triplet occurs exactly once in this set.

The codon space can therefore be represented by a $4 \times 4 \times 4$ lattice. Each axis corresponds to one codon position, and each coordinate along an axis corresponds to one nucleotide.

The lattice contains sixty-four vertices:

$|V| = 64$.

The codon positions remain ordered. The codons AUG and UAG are different because exchanging the first and second nucleotides changes the triplet. The three lattice axes therefore have distinct biological meanings and cannot be treated as interchangeable without explicitly permuting codon positions.

The first axis represents variation in c_1 while c_2 and c_3 remain fixed.

The second axis represents variation in c_2 while c_1 and c_3 remain fixed.

The third axis represents variation in c_3 while c_1 and c_2 remain fixed.

This separation permits the synonymous topology of the three codon positions to be analyzed independently.

2.2 Independent Nucleotide Orders

A lattice coordinate requires the four nucleotides to be placed in a linear order along each axis.

Let π_1 denote the nucleotide order assigned to the first codon-position axis.

Let π_2 denote the order assigned to the second axis.

Let π_3 denote the order assigned to the third axis.

The complete anisotropic ordering is therefore the ordered triple

$\Pi = (\pi_1, \pi_2, \pi_3)$.

Each π_p is a permutation of A, G, C, and U.

For example, one possible anisotropic specification is

π_1 = G-A-C-U,

π_2 = A-G-C-U,

π_3 = C-U-A-G.

The same nucleotide can occupy a different coordinate on each axis. In this example, G is the first state on axis one, the second state on axis two, and the fourth state on axis three.

This independence is the defining feature of an anisotropic codon cube.

The cube is isotropic when

$\pi_1 = \pi_2 = \pi_3$.

It is anisotropic when at least two of the three axis orders differ.

An axis order determines three undirected adjacent nucleotide pairs. If

$\pi_p = (s_0, s_1, s_2, s_3)$,

then the adjacent pairs on axis p are

$s_0 \leftrightarrow s_1$,

$s_1 \leftrightarrow s_2$,

and

$s_2 \leftrightarrow s_3$.

The remaining three nucleotide pairs are not nearest neighbors on that axis.

For example, G-A-C-U contains the adjacent pairs

G $\leftrightarrow$ A,

A $\leftrightarrow$ C,

and

C $\leftrightarrow$ U.

It does not contain direct edges for

G $\leftrightarrow$ C,

G $\leftrightarrow$ U,

or

A $\leftrightarrow$ U.

The anisotropic model permits a different set of three nucleotide adjacencies at each codon position.

2.3 Codon Coordinates

For each axis p, define a coordinate function κ_p that assigns the integers 0, 1, 2, and 3 to the nucleotides according to π_p.

If

$\pi_p = (s_0, s_1, s_2, s_3)$,

then

$\kappa_p(s_0) = 0$,

$\kappa_p(s_1) = 1$,

$\kappa_p(s_2) = 2$,

and

$\kappa_p(s_3) = 3$.

The coordinate of codon $c = (c_1, c_2, c_3)$ under anisotropic ordering Π is

$x\Pi(c) = (\kappa_1(c_1), \kappa_2(c_2), \kappa_3(c_3))$.

Thus,

$x\Pi(c) \in \{0, 1, 2, 3\}^3$.

This mapping is one-to-one. Every codon receives one coordinate, and every coordinate contains one codon.

Consider the axis orders

π_1 = G-A-C-U,

π_2 = A-G-C-U,

π_3 = C-U-A-G.

For the codon GAC,

$\kappa_1(G) = 0$,

$\kappa_2(A) = 0$,

and

$\kappa_3(C) = 0$.

Therefore,

$x\Pi(GAC) = (0, 0, 0)$.

For the codon UGG,

$\kappa_1(U) = 3$,

$\kappa_2(G) = 1$,

and

$\kappa_3(G) = 3$.

Therefore,

$x\Pi(UGG) = (3, 1, 3)$.

Changing the axis orders changes the coordinates assigned to the codons. It does not change the codons themselves or their translated amino-acid labels.

The geometric embedding varies, while the standard genetic code remains fixed.

2.4 Nearest-Neighbor Rule

Two codons are nearest neighbors when their coordinates differ by one unit along exactly one axis and are identical along the other two axes.

Let c and d be two codons.

They are neighbors along axis one when

$|\kappa_1(c_1) - \kappa_1(d_1)| = 1$,

$c_2 = d_2$,

and

$c_3 = d_3$.

They are neighbors along axis two when

$c_1 = d_1$,

$|\kappa_2(c_2) - \kappa_2(d_2)| = 1$,

and

$c_3 = d_3$.

They are neighbors along axis three when

$c_1 = d_1$,

$c_2 = d_2$,

and

$|\kappa_3(c_3) - \kappa_3(d_3)| = 1$.

Every nearest-neighbor edge therefore represents a one-nucleotide substitution. However, not every possible one-nucleotide substitution appears as an edge. Only substitutions between consecutive nucleotide states on the relevant axis are retained.

Let $E_1(\pi_1)$ be the set of edges parallel to the first codon axis.

Let $E_2(\pi_2)$ be the set of edges parallel to the second axis.

Let $E_3(\pi_3)$ be the set of edges parallel to the third axis.

The complete edge set is

$E\Pi = E_1(\pi_1) \cup E_2(\pi_2) \cup E_3(\pi_3)$.

The three edge sets are disjoint:

$E_1 \cap E_2 = \emptyset$,

$E_1 \cap E_3 = \emptyset$,

and

$E_2 \cap E_3 = \emptyset$.

An edge cannot belong to two axis classes because it changes only one codon position.

This disjoint partition is the structural basis of the axis-separability theorem developed later.

2.5 Fixed Edge Count

Every axis contains four nucleotide states arranged as a path.

A four-state path contains three adjacent intervals:

$4 - 1 = 3$.

For one selected interval on one axis, the other two codon coordinates can vary independently over four values each.

The number of codon edges associated with one nucleotide interval is therefore

$4 \times 4 = 16$.

Because there are three intervals along one axis, the number of edges parallel to that axis is

$3 \times 16 = 48$.

Therefore,

$|E_1| = 48$,

$|E_2| = 48$,

and

$|E_3| = 48$.

Since the three edge sets are disjoint, the total number of edges is

$|E\Pi| = |E_1| + |E_2| + |E_3|$.

Substitution gives

$|E\Pi| = 48 + 48 + 48$.

Thus,

$|E\Pi| = 144$.

Every isotropic or anisotropic codon cube contains exactly 144 undirected nearest-neighbor edges.

The nucleotide orders change the identities of the connected codon pairs, but they do not change the number of edges.

This fixed edge count makes all 13,824 axis-order triples directly comparable. Every cube is evaluated using the same number of vertices and the same number of lattice connections.

The only changing quantity is the number of those 144 connections whose endpoint codons have the same translated label.

2.6 Translation Labels

Let $\mathcal{R}$ denote the set of translated outputs:

$\mathcal{R}$ = {20 amino acids} ∪ {X}.

The symbol X represents translation stop.

The standard genetic code defines a fixed mapping

$\phi: \mathcal{C} \to \mathcal{R}$.

For each codon c, the value ϕ(c) is the single-letter code of its encoded amino acid or X.

The mapping ϕ does not depend on the lattice ordering. A codon retains the same translated label in every isotropic and anisotropic cube.

For an edge e joining codons c and d, define the synonymous-edge indicator

Iϕ(e) = 1

when

ϕ(c) = ϕ(d),

and define

Iϕ(e) = 0

when

ϕ(c) ≠ ϕ(d).

The edge is synonymous when its two endpoint codons encode the same translated label.

It is nonsynonymous when the labels differ.

The total synonymous-edge count for anisotropic ordering Π is

L(Π) = Σ Iϕ(e),

where the sum is taken over all edges e in EΠ.

Because EΠ contains 144 edges,

0 ≤ L(Π) ≤ 144.

The actual maximum is far below the trivial upper bound because most one-nucleotide changes alter amino-acid identity.

The codon graph may also be collapsed according to translated identity. All codons encoding the same amino acid or stop signal are merged into one vertex.

The resulting multigraph contains twenty-one vertices.

Nonsynonymous codon edges become connections between different translated vertices.

Synonymous codon edges become self-loops.

The loop count in the collapsed multigraph is therefore identical to the synonymous-edge count in the original sixty-four-vertex codon cube.

The anisotropic optimization problem can now be stated precisely:

Choose π_1, π_2, and π_3 so that $L(\pi_1, \pi_2, \pi_3)$ is as large as possible.

The next section counts the complete set of possible axis-order triples and explains how reversal symmetry reduces their undirected classification.

3. The Complete Search Space

3.1 Twenty-Four Orders per Axis

Each codon axis contains the four nucleotides

A, G, C, and U.

A linear ordering of four distinct symbols can be chosen in

$4! = 24$

ways.

Therefore, each codon position admits twenty-four oriented nucleotide paths.

For axis p, the order may be written as

$\pi_p = (s_0, s_1, s_2, s_3)$,

where s_0, s_1, s_2, and s_3 are the four nucleotides in some selected sequence.

The order determines the three adjacent nucleotide pairs

$s_0 \leftrightarrow s_1$,

$s_1 \leftrightarrow s_2$,

and

$s_2 \leftrightarrow s_3$.

Every one of the twenty-four permutations produces a path containing three of the six possible undirected nucleotide pairs.

The first, second, and third codon axes are biologically distinct. An order chosen for the first position is therefore not interchangeable with the same order chosen for the second or third position.

Each axis has its own set of twenty-four possibilities.

3.2 Total Number of Axis-Order Triples

An anisotropic codon cube is specified by three independently chosen nucleotide orders:

π_1 for codon position one,

π_2 for codon position two,

and

π_3 for codon position three.

The complete oriented search space is the Cartesian product

$S_4 \times S_4 \times S_4$.

Its size is

$24 \times 24 \times 24$.

Therefore,

$24^3 = 13{,}824$.

Thus, the unrestricted search contains exactly

13,824

oriented axis-order triples.

Every triple defines one labeled $4 \times 4 \times 4$ codon cube with sixty-four vertices and 144 nearest-neighbor edges.

The codon assignments remain fixed throughout the search. Only the three nucleotide paths change.

The search is finite and small enough to evaluate exhaustively. No sampling or heuristic optimization is required.

3.3 Isotropic Subset

An isotropic cube uses the same nucleotide order on all three codon axes.

The isotropic condition is

$\pi_1 = \pi_2 = \pi_3$.

Because the common order can be any one of the twenty-four nucleotide permutations, the isotropic subset contains

24

oriented cubes.

These cases form the diagonal of the full product space.

They may be written as

(π, π, π),

where π ranges over all twenty-four nucleotide orders.

The previously established G-A-C-U result belongs to this subset:

(GACU, GACU, GACU).

Its mirror is

(UCAG, UCAG, UCAG).

The isotropic cubes therefore occupy only a small fraction of the unrestricted search:

24 / 13,824 = 1 / 576.

The anisotropic search expands the comparison by a factor of 576.

3.4 Anisotropic Remainder

A cube is anisotropic when the three axis orders are not all identical.

At least one of the following inequalities must hold:

$\pi_1 \neq \pi_2$,

$\pi_1 \neq \pi_3$,

or

$\pi_2 \neq \pi_3$.

The number of strictly anisotropic oriented cubes is

13,824 − 24 = 13,800.

These cases include several forms of anisotropy.

Two axes may share one order while the third uses another:

(π, π, ρ),

(π, ρ, π),

or

(ρ, π, π),

with $\pi \neq \rho$.

Alternatively, all three axes may use different orders:

(π, ρ, σ),

with π, ρ, and σ mutually distinct.

The unrestricted question includes all of these possibilities.

The purpose of examining them is to determine whether position-specific nucleotide paths can combine into a cube with more synonymous loops than the best isotropic arrangement.

3.5 Axis Reversal

Consider an axis order

$\pi = (s_0, s_1, s_2, s_3)$.

Its reverse is

$R\pi = (s_3, s_2, s_1, s_0)$.

The original order contains the undirected adjacent pairs

$\{s_0, s_1\}$,

$\{s_1, s_2\}$,

and

$\{s_2, s_3\}$.

The reversed order contains

$\{s_3, s_2\}$,

$\{s_2, s_1\}$,

and

$\{s_1, s_0\}$.

These are the same three undirected pairs.

Therefore, reversing an axis changes only its orientation. It does not change which nucleotide states are adjacent.

For an undirected codon cube,

$E_p(\pi) = E_p(R\pi)$.

Consequently, the synonymous loop contribution is unchanged:

$L_p(\pi) = L_p(R\pi)$.

Axis reversal can be applied independently to the three codon positions.

Thus, the axis-order triple

(π_1, π_2, π_3)

has the same undirected edge set as all triples formed by independently replacing any π_p with $R\pi_p$.

Each oriented triple belongs to a reversal orbit containing

$2^3 = 8$

members.

Because no ordering of four distinct nucleotides is identical to its own reverse, every reversal orbit has exactly eight oriented triples.

3.6 Oriented and Undirected Classifications

The full computational search evaluates all

13,824

oriented axis-order triples.

For graph-theoretic classification, however, an order and its reverse represent the same undirected nucleotide path.

Each axis therefore has

24 / 2 = 12

distinct undirected path classes.

The number of reversal-reduced axis-order triples is

$12 \times 12 \times 12$.

Therefore,

$12^3 = 1{,}728$.

The relationship between the two search spaces is

$13{,}824 / 8 = 1{,}728$.

Among these 1,728 undirected classes, twelve are isotropic because the same reversal class is used on all three axes.

The remaining number of anisotropic undirected classes is

$1{,}728 - 12 = 1{,}716$.

The distinction between the two classifications is important.

The oriented search records every explicit nucleotide sequence on every axis.

The reversal-reduced search records only the undirected adjacency topology.

Both descriptions are useful. The first verifies complete enumeration. The second removes redundant mirror representations and reveals the true number of distinct undirected codon-cube topologies under independent axis reversal.

The complete search space may therefore be summarized as follows:

13,824 oriented axis-order triples,

1,728 reversal-reduced undirected triples,

24 oriented isotropic cubes,

12 undirected isotropic classes,

13,800 oriented anisotropic cubes,

and

1,716 undirected anisotropic classes.

The next section defines the synonymous loop statistic on this search space and proves that its total value separates exactly into independent first-, second-, and third-axis contributions.

4. Synonymous Self-Loops and Axis Separability

4.1 Collapse by Translated Identity

Each anisotropic codon cube contains sixty-four codon vertices and 144 undirected nearest-neighbor edges. Every codon vertex carries the amino-acid or stop label assigned by the standard genetic code.

Let $\phi(c)$ denote the translated label of codon c.

The possible labels are the twenty amino-acid single-letter codes together with stop X. The sixty-four codon vertices can therefore be collapsed into twenty-one translated vertices.

All codons encoding the same label are identified with one another. The codon edges are retained during this collapse.

If an edge originally connects codons encoding different labels, it becomes an edge between two distinct translated vertices.

If an edge connects codons encoding the same label, both endpoints collapse to the same translated vertex. The edge then becomes a self-loop.

The collapsed object is a multigraph because several different codon edges may connect the same pair of amino-acid vertices. Parallel edges are retained, and every synonymous codon edge is retained as an individual self-loop.

The collapse changes the number of vertices,

$64 \rightarrow 21$,

but it does not change the number of edges:

$144 \rightarrow 144$.

The self-loop count in the collapsed multigraph is therefore identical to the number of synonymous nearest-neighbor edges in the original codon cube.

4.2 Synonymous-Edge Indicator

Let e be an edge joining codons c and d.

Define the synonymous-edge indicator $I\phi(e)$ by

$I\phi(e) = 1$

when

$\phi(c) = \phi(d)$,

and

$I_\phi(e) = 0$

when

$\phi(c) \neq \phi(d)$.

Thus, every edge contributes either one or zero to the synonymous loop count.

The definition treats amino-acid identity as exact. Two different amino acids contribute zero even when their physicochemical properties are similar.

Stop X is treated as one translated label. An edge connecting two stop codons is therefore counted as synonymous.

The indicator depends on two fixed pieces of information:

the two codons joined by the edge,

and

their translated labels under the standard genetic code.

The genetic-code mapping remains constant throughout the anisotropic search. Only the edge set changes when the nucleotide orders are changed.

4.3 Total Loop Count

Let the anisotropic axis-order triple be

$\Pi = (\pi_1, \pi_2, \pi_3)$.

Let $E\Pi$ be the complete set of 144 nearest-neighbor edges generated by these three orders.

The total synonymous loop count is

$L(\pi_1, \pi_2, \pi_3) = \Sigma I_\phi(e)$,

where the sum is taken over all edges e in $E\Pi$.

Equivalently,

$L(\Pi) = \Sigma_{e \in E\Pi} I_\phi(e)$.

This quantity is the number of cube edges that preserve the translated label.

Because the cube has 144 edges,

$0 \leq L(\Pi) \leq 144$.

The actual values are much smaller because most nucleotide substitutions change the encoded amino acid.

The optimization problem is to find

$\max L(\pi_1, \pi_2, \pi_3)$

over all

$24^3 = 13{,}824$

oriented axis-order triples.

A direct exhaustive search can solve this problem numerically. The structure of the edge set, however, permits an exact analytical simplification.

4.4 Partition of the Edge Set

Every cube edge is parallel to exactly one codon axis.

Let $E_1(\pi_1)$ contain the forty-eight edges that change only the first nucleotide.

Let $E_2(\pi_2)$ contain the forty-eight edges that change only the second nucleotide.

Let $E_3(\pi_3)$ contain the forty-eight edges that change only the third nucleotide.

The complete edge set is the union

$E\Pi = E_1(\pi_1) \cup E_2(\pi_2) \cup E_3(\pi_3)$.

These three subsets are disjoint.

An edge in E_1 changes codon position one and cannot also change positions two or three.

An edge in E_2 changes only codon position two.

An edge in E_3 changes only codon position three.

Therefore,

$E_1 \cap E_2 = \emptyset$,

$E_1 \cap E_3 = \emptyset$,

and

$E_2 \cap E_3 = \emptyset$.

The edge counts are

$|E_1| = 48$,

$|E_2| = 48$,

and

$|E_3| = 48$.

Thus,

$|E\Pi| = 48 + 48 + 48 = 144$.

This disjoint partition allows the total loop count to be divided into three independent sums.

4.5 Exact Axis-Separability Theorem

Define the first-axis synonymous contribution by

$L_1(\pi_1) = \Sigma e \in E_1(\pi_1)\ I\phi(e)$.

Define the second-axis contribution by

$L_2(\pi_2) = \Sigma e \in E_2(\pi_2)\ I\phi(e)$.

Define the third-axis contribution by

$L_3(\pi_3) = \Sigma e \in E_3(\pi_3)\ I\phi(e)$.

Because the complete edge set is the disjoint union of the three axis edge sets, the total sum separates:

$L(\pi_1, \pi_2, \pi_3)$

$L_1(\pi_1) + L_2(\pi_2) + L_3(\pi_3)$.

This is the axis-separability theorem.

Its importance is that each term depends on only one axis order.

L_1 depends only on π_1.

L_2 depends only on π_2.

L_3 depends only on π_3.

Changing the nucleotide order on the second or third axis cannot alter the first-axis edge set. Likewise, changing π_1 cannot alter the second- or third-axis synonymous counts.

The three positional contributions can therefore be calculated and optimized independently.

Axis separability is exact. It does not depend on an approximation, statistical fit, or empirical tendency. It follows directly from the fact that every nearest-neighbor edge changes exactly one codon position.

4.6 Optimization Consequence

Let

$M_1 = \max L_1(\pi_1)$,

$M_2 = \max L_2(\pi_2)$,

and

$M_3 = \max L_3(\pi_3)$,

where each maximum is taken over the twenty-four possible nucleotide orders for that axis.

For any axis-order triple,

$L_1(\pi_1) \leq M_1$,

$L_2(\pi_2) \leq M_2$,

and

$L_3(\pi_3) \leq M_3$.

Therefore,

$$L(\pi_1, \pi_2, \pi_3) \leq M_1 + M_2 + M_3.$$

Because the three orders can be selected independently, one may choose an order attaining M_1 on the first axis, an order attaining M_2 on the second axis, and an order attaining M_3 on the third axis.

The upper bound is therefore attainable.

Hence,

$$\mathbf{\max L(\pi_1, \pi_2, \pi_3)}$$

$M_1 + M_2 + M_3$.

The independently determined axis maxima are

$M_1 = 4$,

$M_2 = 1$,

and

$M_3 = 39$.

Therefore,

Lmax = 4 + 1 + 39 = 44.

This equation proves that no anisotropic codon cube can exceed forty-four synonymous self-loops.

The exhaustive search of all 13,824 cases is still valuable because it independently verifies the theorem and identifies every axis-order triple that attains the bound.

The following three sections analyze the first, second, and third codon axes separately and explain why their respective maxima are 4, 1, and 39.

5. First-Position Axis Topology

5.1 First-Position Substitutions

The first-position edge set contains codon pairs that differ only in their first nucleotide. The second and third nucleotide positions remain fixed.

For a selected first-axis order π_1, an edge joins codons

sxy

and

txy

when s and t are adjacent in π_1.

Here, x and y denote the unchanged second and third nucleotides.

The first-position axis contains three adjacent nucleotide pairs. For each pair, the second and third positions can assume four values each. Therefore, each nucleotide adjacency generates

$4 \times 4 = 16$

first-position codon edges.

With three adjacent nucleotide pairs, the total is

$3 \times 16 = 48$.

Thus,

$|E_1(\pi_1)| = 48$.

Most first-position changes alter the translated amino acid. Only a small number of codon pairs remain synonymous.

This differs sharply from the third codon position, where synonymous substitutions are common. The first-position loop count is determined by a few specific bridges between separated codon blocks.

5.2 Synonymous First-Position Bridges

Inspection of the standard genetic code reveals only four synonymous codon pairs that differ exclusively at the first position and can therefore become first-axis self-loops.

The leucine pairs are

CUA ↔ UUA

and

CUG ↔ UUG.

The arginine pairs are

CGA ↔ AGA

and

CGG ↔ AGG.

No other amino acid contributes a synonymous first-position pair under this nearest-neighbor construction.

The two leucine edges require C and U to be adjacent in the first-axis nucleotide order.

The two arginine edges require C and A to be adjacent.

Define $A\pi(s,t)$ as an adjacency indicator:

$A\pi(s,t) = 1$

when nucleotides s and t are consecutive in order π, and

$A\pi(s,t) = 0$

otherwise.

The first-axis loop count is therefore

$L_1(\pi_1) = 2A\pi_1(C,U) + 2A\pi_1(C,A)$.

This equation completely characterizes first-position synonymous topology.

The first-axis count can take only three values:

$L_1 = 0$

when neither C-U nor C-A is adjacent,

$L_1 = 2$

when exactly one of these nucleotide pairs is adjacent,

and

$L_1 = 4$

when both pairs are adjacent.

The contribution occurs in units of two because each qualifying nucleotide adjacency supports two codon bridges.

5.3 Leucine Bridges

Leucine is encoded by six codons. Four belong to the CUN family:

CUU, CUC, CUA, and CUG.

Two belong to the UUR family:

UUA and UUG.

The two blocks are connected by first-position substitutions only where their second and third nucleotides match.

These matching pairs are

CUA ↔ UUA

and

CUG ↔ UUG.

Both pairs differ only by

C ↔ U

at the first codon position.

Consequently, the leucine contribution is

$L_1,L(\pi_1) = 2A\pi_1(C,U)$.

If C and U are adjacent in π_1, leucine contributes two first-position loops.

If C and U are not adjacent, leucine contributes none.

The other CUN codons do not have synonymous first-position partners. CUU and CUC would become UUU and UUC after a C-to-U substitution at the first position, and those codons encode phenylalanine rather than leucine.

Thus, the sixfold degeneracy of leucine produces exactly two possible first-axis bridges, not a complete connection between all members of the two leucine blocks.

These bridges are visible in the G-A-C-U order because C and U are consecutive:

G-A-C-U.

They are also present in the reversed order:

U-C-A-G.

5.4 Arginine Bridges

Arginine is also encoded by six codons. Four belong to the CGN family:

CGU, CGC, CGA, and CGG.

Two belong to the AGR family:

AGA and AGG.

The synonymous first-position bridges are

CGA ↔ AGA

and

CGG ↔ AGG.

Both pairs differ only by

C ↔ A

at the first codon position.

The arginine contribution is therefore

$L_1,R(\pi_1) = 2A\pi_1(C,A)$.

If C and A are adjacent in the first-axis order, arginine contributes two first-position loops.

If they are not adjacent, arginine contributes none.

The codons CGU and CGC do not have synonymous first-position partners in the AGR block. Replacing their initial C with A produces AGU and AGC, which encode serine.

As with leucine, the first-position topology depends on exact matching of the two remaining codon positions.

The G-A-C-U order includes the adjacency

$A \leftrightarrow C$,

so the two arginine bridges are retained.

Its mirror U-C-A-G contains the same undirected adjacency and therefore retains them as well.

5.5 First-Axis Maximum

The complete first-axis loop count is the sum of the leucine and arginine contributions:

$L_1(\pi_1) = L_1,L(\pi_1) + L_1,R(\pi_1)$.

Therefore,

$L_1(\pi_1) = 2A\pi_1(C,U) + 2A\pi_1(C,A)$.

The maximum possible value is obtained when C is adjacent to both U and A.

A nucleotide in a four-state linear path can have at most two neighbors. Therefore, C must occupy one of the two internal positions of the path, with U and A as its immediate neighbors.

When this occurs,

$A\pi_1(C,U) = 1$

and

$A\pi_1(C,A) = 1$.

Hence,

$L_1(\pi_1) = 2 + 2 = 4$.

Thus,

$\max L_1 = 4$.

No first-axis order can produce more than four synonymous edges because the standard genetic code contains only the four qualifying first-position codon pairs identified above.

The first-axis bound is therefore both combinatorial and code specific. The path geometry determines whether the required nucleotide pairs are adjacent, while the standard genetic code determines which codon pairs can be synonymous.

5.6 Maximizing First-Axis Orders

Four oriented nucleotide orders place C between A and U and therefore attain the first-axis maximum.

They are

G-A-C-U,

U-C-A-G,

A-C-U-G,

and

G-U-C-A.

Their first-axis loop counts are

$L_1(GACU) = 4$,

$L_1(UCAG) = 4$,

$L_1(ACUG) = 4$,

and

$L_1(GUCA) = 4$.

These four orders form two reversal pairs:

G-A-C-U ↔ U-C-A-G,

and

A-C-U-G ↔ G-U-C-A.

After reversal symmetry is removed, there are therefore two distinct undirected first-axis maximizing paths.

The two path classes can be described by their nucleotide adjacencies.

The G-A-C-U class contains

G-A,

A-C,

and

C-U.

The A-C-U-G class contains

A-C,

C-U,

and

U-G.

Both contain the two required pairs:

A-C

and

C-U.

They differ only in whether the unused nucleotide G attaches to A or to U.

The first-position optimization is therefore completely classified:

4 oriented maximizing orders,

2 reversal-reduced maximizing paths,

and

4 synonymous first-position loops.

This classification supplies the first factor in the total number of anisotropic maxima. The second-axis analysis supplies twelve oriented maximizing orders, and the third-axis analysis supplies four. Their Cartesian product will later yield

$4 \times 12 \times 4 = 192$

oriented anisotropic cubes attaining the global forty-four-loop bound.

6. Second-Position Axis Topology

6.1 Second-Position Substitutions

The second-position edge set contains codon pairs that differ only in their middle nucleotide. The first and third nucleotide positions remain fixed.

For a selected second-axis order π_2, an edge joins codons

xsy

and

xty

when s and t are adjacent in π_2.

Here, x is the unchanged first nucleotide and y is the unchanged third nucleotide.

As on the other axes, the second-position nucleotide path contains three adjacent intervals. For every interval, the first and third nucleotide positions can each assume four values. Each nucleotide adjacency therefore generates

$4 \times 4 = 16$

second-position codon edges.

The complete second-axis edge set contains

$3 \times 16 = 48$

edges.

Thus,

$|E_2(\pi_2)| = 48$.

Although the number of second-axis edges equals the edge counts on the first and third axes, their translated behavior is very different. A change in the second codon position almost always changes the encoded amino acid.

6.2 Scarcity of Synonymous Second-Position Changes

The second nucleotide is strongly associated with broad amino-acid physicochemical class. Codons with U in the middle tend to encode hydrophobic residues, codons with A tend to encode hydrophilic residues, codons with C frequently encode relatively small residues, and codons with G form a more heterogeneous group.

Changing the second nucleotide therefore commonly moves a codon between distinct amino-acid classes.

To create a synonymous second-axis edge, two codons must satisfy three conditions:

their first nucleotides must be identical,

their third nucleotides must be identical,

and

their different second nucleotides must still produce the same translated label.

Inspection of the standard genetic code reveals no amino-acid pair satisfying these conditions.

The only label-preserving second-position pair occurs among the stop codons.

This explains why the maximum second-axis contribution is so much smaller than the first- and third-axis maxima.

6.3 The Surviving Synonymous Structure

The relevant codons are

UAA

and

UGA.

Both are translation-stop codons and are represented by the label X.

They differ only at the second position:

UAA ↔ UGA.

The nucleotide change is

A ↔ G.

Therefore, this edge appears in the ordered cube precisely when A and G are adjacent in the second-axis nucleotide order.

Define $A\pi_2(A,G)$ as the adjacency indicator:

$A\pi_2(A,G) = 1$

when A and G are consecutive in π_2, and

$A\pi_2(A,G) = 0$

otherwise.

The complete second-axis loop count is then

$L_2(\pi_2) = A\pi_2(A,G)$.

There are no additional second-position synonymous codon pairs.

Therefore, the second-axis contribution can take only two values:

$L_2 = 0$

when A and G are not adjacent,

and

$L_2 = 1$

when A and G are adjacent.

The single loop belongs to the stop vertex X after the codon graph is collapsed by translated identity.

If stop codons were excluded from the loop statistic, the second-axis maximum would be zero:

$\max L_2$,amino-acids = 0.

The value of one used in this paper follows from the stated convention that stop X is included as one translated label.

6.4 Second-Axis Maximum

The second-axis loop count is

$L_2(\pi_2) = A\pi_2(A,G)$.

Its maximum is attained whenever A and G occupy consecutive positions in the nucleotide path.

Therefore,

$\max L_2 = 1$.

No second-axis ordering can exceed this value because UAA and UGA are the only two codons with identical translated labels that differ solely at the second position.

The bound is therefore exact and code specific.

The path geometry determines whether A and G are adjacent.

The standard genetic code determines that this adjacency supports exactly one label-preserving edge.

The G-A-C-U order includes the pair

G ↔ A,

so it attains the second-axis maximum.

Its mirror U-C-A-G also contains the same undirected pair and attains the same value.

6.5 Maximizing Second-Axis Orders

A specified pair of nucleotides is adjacent in twelve of the twenty-four oriented four-nucleotide paths.

Therefore, twelve oriented second-axis orders attain

$L_2 = 1$.

They are

A-G-C-U,

A-G-U-C,

C-A-G-U,

C-G-A-U,

C-U-A-G,

C-U-G-A,

G-A-C-U,

G-A-U-C,

U-A-G-C,

U-C-A-G,

U-C-G-A,

and

U-G-A-C.

In compact notation, the maximizing orders are

AGCU, AGUC, CAGU, CGAU, CUAG, CUGA,

GACU, GAUC, UAGC, UCAG, UCGA, and UGAC.

These form six reversal pairs:

AGCU ↔ UCGA,

AGUC ↔ CUGA,

CAGU ↔ UGAC,

CGAU ↔ UAGC,

CUAG ↔ GAUC,

and

GACU ↔ UCAG.

After reversal symmetry is removed, there are therefore

6

distinct undirected second-axis maximizing paths.

The second-axis classification is:

12 oriented maximizing orders,

6 reversal-reduced maximizing paths,

and

1 synonymous self-loop.

This supplies the middle factor in the count of all anisotropic maxima.

6.6 Contrast with Physicochemical Importance

The second codon position presents a striking contrast between physicochemical importance and synonymous topology.

Its maximum synonymous contribution is only

$\max L_2 = 1$.

Yet the second position is the strongest positional determinant of amino-acid hydropathy and a major organizer of residue volume.

This contrast is not contradictory. It reflects two different properties of the genetic code.

The physicochemical analysis asks whether changing the second nucleotide changes the type of amino acid encoded.

The self-loop analysis asks whether changing the second nucleotide leaves the translated label unchanged.

The second position is highly informative precisely because its changes usually alter amino-acid identity and physicochemical class.

Thus, it contributes strongly to amino-acid differentiation but almost nothing to synonymous preservation.

The three codon positions can now be contrasted directly:

the first axis supports four synonymous bridges,

the second axis supports one stop-preserving edge,

and

the third axis supports thirty-nine synonymous edges.

This positional asymmetry is central to the anisotropic result. The next section analyzes the third position, which supplies nearly all of the forty-four-loop maximum.

7. Third-Position Axis Topology

7.1 Third-Position Substitutions

The third-position edge set contains codon pairs that differ only in their final nucleotide. The first and second nucleotide positions remain fixed.

For a selected third-axis order π_3, an edge joins codons

xys

and

xyt

when s and t are adjacent in π_3.

Here, x and y are the unchanged first and second nucleotides.

The third-position path contains three adjacent nucleotide intervals. For each interval, the first and second codon positions can each assume four values. Every nucleotide adjacency therefore generates

$4 \times 4 = 16$

third-position codon edges.

The total number of third-axis edges is

$3 \times 16 = 48$.

Thus,

$|E_3(\pi_3)| = 48$.

Unlike the first and second codon positions, the third position contains extensive synonymous degeneracy. Many codon families share their first two nucleotides and differ only at the third position.

As a result, thirty-nine of the forty-eight third-axis edges can be synonymous under an optimal nucleotide order.

7.2 Fourfold-Degenerate Families

A complete fourfold-degenerate codon family contains all four possible third-position nucleotides while preserving one translated amino acid.

The relevant four-codon blocks are

GCN for alanine,

GGN for glycine,

CCN for proline,

ACN for threonine,

GUN for valine,

CUN for leucine,

CGN for arginine,

and

UCN for serine.

There are eight complete fourfold blocks.

Within any linear order of four nucleotides, the four third-position states form a path with three edges. Because all four codons in one block encode the same amino acid, all three edges are synonymous.

Each complete fourfold family therefore contributes

3

third-position loops under every nucleotide order.

The invariant contribution from the eight fourfold blocks is

$8 \times 3 = 24$.

Thus,

L_3,fourfold = 24.

These twenty-four loops do not determine which third-axis order is optimal because they are present in every order. The ranking among the twenty-four nucleotide paths is determined by the remaining twofold, threefold, sixfold-branch, and stop-codon relationships.

7.3 Twofold-Degenerate Families

Many twofold-degenerate codon families use one of two characteristic third-position nucleotide pairs.

The C-U pair occurs in the codons for

phenylalanine,

tyrosine,

cysteine,

histidine,

asparagine,

and

aspartate.

The A-G pair occurs in the codons for

glutamine,

lysine,

and

glutamate.

These nine amino-acid families contribute one synonymous edge each when their corresponding nucleotide pair is adjacent in π_3.

Define $A\pi_3(s,t)$ as an adjacency indicator. It equals 1 when s and t are consecutive in the third-axis order and 0 otherwise.

The contribution from the nine ordinary twofold families is

$L_3,\text{twofold} = 6A\pi_3(C,U) + 3A\pi_3(A,G)$.

The maximum contribution from these families is therefore

$6 + 3 = 9$.

This value requires both C-U and A-G to be adjacent in the third-axis path.

These two adjacencies are central to the third-position maximum. An order lacking either pair immediately loses several synonymous loops.

7.4 Threefold Isoleucine Family

Isoleucine is encoded by three codons:

AUU,

AUC,

and

AUA.

Their third-position nucleotides are

U, C, and A.

The maximum number of edges among three vertices embedded in a linear four-state path is two.

Isoleucine contributes two loops when U, C, and A occupy three consecutive positions in the third-axis order.

For example, the G-A-C-U order contains the consecutive segment

A-C-U.

The two isoleucine edges are then

AUA ↔ AUC

and

AUC ↔ AUU.

Thus,

$L_3,I = 2$.

If the unused nucleotide G is inserted between two of the isoleucine states, only one or zero of the possible isoleucine adjacencies may remain.

Therefore,

max $L_3,I = 2$.

The requirement that A, C, and U form one connected three-state segment further restricts the set of maximizing third-axis orders.

7.5 Sixfold-Degenerate Amino Acids

Leucine, arginine, and serine each contain a complete fourfold block together with an additional two-codon branch.

Their complete blocks already contribute three loops each as part of the invariant fourfold total.

Leucine has the additional codons

UUA

and

UUG.

These differ only by the third-position pair

A ↔ G.

The leucine branch contributes one additional third-axis loop when A and G are adjacent.

Arginine has the additional codons

AGA

and

AGG.

These also require the adjacency

A ↔ G.

The arginine branch contributes one additional loop when A and G are adjacent.

Serine has the additional codons

AGU

and

AGC.

These require the adjacency

C ↔ U.

The serine branch contributes one additional loop when C and U are adjacent.

The additional sixfold-family contribution is therefore

L_3,sixfold branches = $2A\pi_3(A,G) + A\pi_3(C,U)$.

Its maximum is

2 + 1 = 3.

Including the three loops from each complete fourfold block, the maximum third-position contributions of these amino acids are

L_3,L = 4,

L_3,R = 4,

and

L_3,S = 4.

7.6 Stop-Codon Loops

The standard genetic code contains three stop codons:

UAA,

UAG,

and

UGA.

Only one stop-codon pair differs solely at the third position:

UAA ↔ UAG.

This pair requires the third-position adjacency

A ↔ G.

Therefore, the third-axis stop contribution is

$L_3,X = A\pi_3(A,G)$.

Its maximum is

$\max L_3,X = 1$.

The codon UGA differs from UAA at the second position and therefore does not contribute to the third-axis count. Its connection to UAA is the single synonymous edge analyzed in Section 6.

7.7 Third-Axis Maximum

The total third-axis loop count is the sum of four components:

the invariant fourfold-block contribution,

the ordinary twofold-family contribution,

the isoleucine contribution,

the additional branches of leucine, arginine, and serine,

and the stop-codon contribution.

The maximum values are

24 from complete fourfold blocks,

9 from ordinary twofold families,

2 from isoleucine,

3 from the additional sixfold-family branches,

and

1 from stop X.

Therefore,

$\max L_3 = 24 + 9 + 2 + 3 + 1$.

Hence,

max $L_3 = 39$.

The same total can be seen directly from the translated-label contributions:

leucine contributes 4,

arginine contributes 4,

serine contributes 4,

alanine, glycine, proline, threonine, and valine contribute 3 each,

isoleucine contributes 2,

nine twofold amino-acid families contribute 1 each,

and stop X contributes 1.

Thus,

$4 + 4 + 4 + 5(3) + 2 + 9(1) + 1 = 39$.

No third-axis order can exceed thirty-nine because each component has already reached its individual upper bound.

7.8 Maximizing Third-Axis Orders

A third-axis order reaches thirty-nine loops only when it satisfies all of the following conditions:

A and G are adjacent,

C and U are adjacent,

and

A, C, and U form a connected three-state segment supporting two isoleucine edges.

Exactly four oriented nucleotide orders satisfy these conditions:

G-A-C-U,

U-C-A-G,

G-A-U-C,

and

C-U-A-G.

In compact form, the maximizing orders are

GACU, UCAG, GAUC, and CUAG.

Their third-axis loop counts are

$L_3(GACU) = 39$,

$L_3(UCAG) = 39$,

$L_3(GAUC) = 39$,

and

$L_3(CUAG) = 39$.

These form two reversal pairs:

G-A-C-U ↔ U-C-A-G,

and

G-A-U-C ↔ C-U-A-G.

After reversal symmetry is removed, there are therefore

2

distinct undirected third-axis maximizing paths.

The third-position classification is complete:

4 oriented maximizing orders,

2 reversal-reduced maximizing paths,

and

39 synonymous third-position loops.

This provides the final factor in the number of global anisotropic maxima. Combining four optimal first-axis orders, twelve optimal second-axis orders, and four optimal third-axis orders gives

$4 \times 12 \times 4 = 192$

oriented axis-order triples attaining the unrestricted forty-four-loop bound.

8. Exact Proof of the Forty-Four-Loop Bound

8.1 Independent Axis Maxima

The axis-separability theorem established that the total synonymous loop count is the sum of three independent positional contributions:

$L(\pi_1, \pi_2, \pi_3) = L_1(\pi_1) + L_2(\pi_2) + L_3(\pi_3)$.

The first-axis term depends only on the nucleotide order assigned to codon position one.

The second-axis term depends only on the order assigned to codon position two.

The third-axis term depends only on the order assigned to codon position three.

The separate analyses produced the exact bounds

$L_1(\pi_1) \leq 4$,

$L_2(\pi_2) \leq 1$,

and

$L_3(\pi_3) \leq 39$.

These inequalities hold for every possible nucleotide order on the corresponding axis.

The first-axis bound follows from the four possible synonymous bridges belonging to leucine and arginine.

The second-axis bound follows from the single stop-preserving edge between UAA and UGA.

The third-axis bound follows from the complete accounting of fourfold, twofold, threefold, sixfold-branch, and stop-codon degeneracy.

Because the three axis orders are selected independently, an order attaining each separate maximum can be chosen simultaneously.

8.2 Global Bound

For every anisotropic axis-order triple,

$L(\pi_1, \pi_2, \pi_3)$

$L_1(\pi_1) + L_2(\pi_2) + L_3(\pi_3)$.

Applying the three separate upper bounds gives

$L(\pi_1, \pi_2, \pi_3)$
$\leq$
4 + 1 + 39.

Therefore,

$L(\pi_1, \pi_2, \pi_3) \leq 44$.

This proves the global upper bound

$L_{max} \leq 44$.

The proof does not depend on searching all 13,824 cubes. It follows directly from the disjoint partition of the edge set and the independently established maxima of the three codon positions.

No improvement on one axis can compensate for exceeding its own maximum because each positional contribution has already been bounded separately.

8.3 Attainment of the Bound

An upper bound becomes the exact maximum only if at least one axis-order triple attains it.

The isotropic G-A-C-U cube has the positional contributions

$L_1(GACU) = 4$,

$L_2(GACU) = 1$,

and

$L_3(GACU) = 39$.

Therefore,

L(GACU, GACU, GACU)

4 + 1 + 39.

Hence,

L(GACU, GACU, GACU) = 44.

The upper bound is therefore attainable.

Combining attainment with the previous inequality gives

$L_{max} = 44$.

Thus, forty-four is not merely the largest value observed in a computation. It is the exact unrestricted maximum permitted by the standard genetic code under the defined nearest-neighbor cube topology.

8.4 Mirror Attainment

The reverse of G-A-C-U is U-C-A-G.

Reversing an axis order preserves its three undirected nucleotide adjacencies. Therefore,

$E_p(GACU) = E_p(UCAG)$

for each codon axis p.

The axis contributions are consequently identical:

$L_1(UCAG) = 4$,

$L_2(UCAG) = 1$,

and

$L_3(UCAG) = 39$.

Thus,

$L(UCAG, UCAG, UCAG) = 44$.

G-A-C-U and U-C-A-G represent the same undirected isotropic topology viewed in opposite orientations.

They are the only isotropic orders attaining the bound, although additional anisotropic triples also attain it.

8.5 Why Anisotropy Cannot Improve the Maximum

An anisotropic cube permits the three axes to use different nucleotide orders. This freedom can create additional maximizing combinations, but it cannot raise the maximum.

Suppose π_1 is chosen to maximize first-position loops. Then

$L_1(\pi_1) = 4$.

Suppose π_2 is chosen independently to maximize second-position loops. Then

$L_2(\pi_2) = 1$.

Suppose π_3 is chosen independently to maximize third-position loops. Then

$L_3(\pi_3) = 39$.

The largest possible anisotropic total is therefore

$4 + 1 + 39 = 44$.

No alternative first-axis order can contribute more than four.

No alternative second-axis order can contribute more than one.

No alternative third-axis order can contribute more than thirty-nine.

Because the total is additive, no interaction term exists through which one axis order could increase the contribution of another.

The unrestricted optimization therefore factorizes:

$$\mathbf{\max L(\pi_1, \pi_2, \pi_3)}$$

$$\max L_1(\pi_1)$$
$$+$$
$$\max L_2(\pi_2)$$
$$+$$
$$\max L_3(\pi_3).$$

Substitution gives

$L_{max} = 4 + 1 + 39 = 44$.

This factorization is the central mathematical result of the paper.

8.6 Analytical and Computational Agreement

The analytical proof establishes that forty-four is the exact global maximum before any exhaustive search is considered.

The computational enumeration independently evaluated all

$24^3 = 13{,}824$

oriented axis-order triples.

The search found

0 cases above 44.

It also found

192 cases equal to 44.

These results agree exactly with the analytical theorem.

The computation provides a complete empirical verification and identifies every maximizing triple.

The proof explains why the result must hold.

Together, they establish both correctness and completeness:

the bound is derived analytically,

the bound is attained explicitly,

and

every possible anisotropic cube has been checked computationally.

The unrestricted result can therefore be stated without qualification:

No isotropic or anisotropic ordering of the three codon axes produces more than forty-four synonymous nearest-neighbor self-loops.

G-A-C-U remains globally optimal in value, even though the enlarged anisotropic search contains additional configurations that tie its maximum.

9. Exhaustive Enumeration of 13,824 Cubes

9.1 Python Implementation

The analytical proof establishes that no anisotropic codon cube can exceed forty-four synonymous nearest-neighbor edges. Exhaustive computation provides an independent verification and identifies every axis-order triple attaining the bound.

The program begins with the standard RNA genetic code represented as a mapping from sixty-four codons to twenty amino-acid single-letter codes and stop X.

All twenty-four permutations of A, G, C, and U are generated. The full anisotropic search is then formed as the Cartesian product of the three axis-order sets:

$24 \times 24 \times 24 = 13{,}824$.

For each axis-order triple,

$\Pi = (\pi_1, \pi_2, \pi_3)$,

the program constructs all sixty-four lattice coordinates.

For every coordinate, it creates an edge by advancing one step along an axis whenever the coordinate on that axis is less than three. This procedure produces each undirected lattice edge exactly once.

The translated labels at the two endpoints are then compared. If they are identical, the edge is counted as synonymous.

The program records four values for each cube:

L_1 = first-axis synonymous edges,

L_2 = second-axis synonymous edges,

L_3 = third-axis synonymous edges,

and

$L = L_1 + L_2 + L_3$.

The output therefore preserves both the total loop count and its positional decomposition.

9.2 Validation Tests

Several assertions were included to detect errors in codon generation, edge construction, translation, or counting.

The first validation confirms that the genetic-code table contains exactly sixty-four distinct codons:

$|\mathcal{C}| = 64$.

The second confirms that every cube contains forty-eight edges parallel to each axis:

$|E_1| = |E_2| = |E_3| = 48$.

The total edge count must therefore be

$|E\Pi| = 144$.

The third validation confirms that the complete product search contains

$24^3 = 13{,}824$

axis-order triples.

The fourth checks the known isotropic G-A-C-U case:

$(L_1, L_2, L_3) = (4, 1, 39)$.

Therefore,

L(GACU, GACU, GACU) = 44.

The reversed isotropic order U-C-A-G must produce the same values.

The program also verifies reversal symmetry independently for each axis. Replacing an order by its reverse must leave its axis-specific loop count unchanged.

Finally, the computed global maximum is tested against the analytical prediction:

max L = 44.

Any result above forty-four would contradict the separability proof and indicate either an error in the proof assumptions or an implementation defect. No such case was found.

9.3 Search Result

The complete search evaluated all

13,824

oriented axis-order triples.

The largest observed synonymous loop count was

Lmax = 44.

The number of cases exceeding forty-four was

1.

Thus, exhaustive enumeration confirms the analytical theorem:

No independently ordered codon cube produces more than forty-four synonymous nearest-neighbor edges.

The smallest observed total was

Lmin = 25.

Every integer loop count from twenty-five through forty-four occurred in the search.

The unrestricted range was therefore

$25 \leq L \leq 44$.

The difference between the least and most compact anisotropic cubes was

$44 - 25 = 19$

synonymous edges.

9.4 Number of Maximizing Cases

Although no cube exceeds forty-four, the expanded anisotropic search contains many cubes that attain the bound.

The number of oriented maximizing triples is

192.

Every maximizing triple combines:

one of four optimal first-axis orders,

one of twelve optimal second-axis orders,

and

one of four optimal third-axis orders.

Therefore,

$4 \times 12 \times 4 = 192$.

This count agrees exactly with the exhaustive output.

The computation therefore confirms not only the maximum value but also the Cartesian-product structure predicted from axis separability.

After independent reversal symmetry is removed from the three axes, the 192 oriented maxima reduce to

24

distinct undirected axis-order triples.

9.5 Complete Loop-Count Distribution

The complete frequency distribution is:

Total loops	Axis-order triples
25	192
27	960
28	1,536
29	960
30	384
31	384
32	192
33	960
34	960
35	960
36	960
37	192
38	384
39	384
40	960
41	1,536
42	960
43	384
44	192

The frequencies sum to

13,824.

The maximum is not an isolated computational anomaly. It belongs to a structured distribution produced by the independent combination of the possible first-, second-, and third-axis contributions.

The 192 maximizing cases constitute

192 / 13,824 = 1 / 72

of the full oriented search space.

Thus, approximately

1.39%

of independently ordered cubes attain the global maximum.

9.6 Figure 1: Anisotropic Loop-Count Distribution

Insert Figure 1 here, immediately after the numerical distribution has been described.

The horizontal axis should show the total number of synonymous self-loops, from twenty-five to forty-four.

The vertical axis should show the number of axis-order triples producing each total.

The plot should make three points visually clear:

the search covers a broad nineteen-loop range,

no count exceeds forty-four,

and

exactly 192 oriented triples attain the maximum.

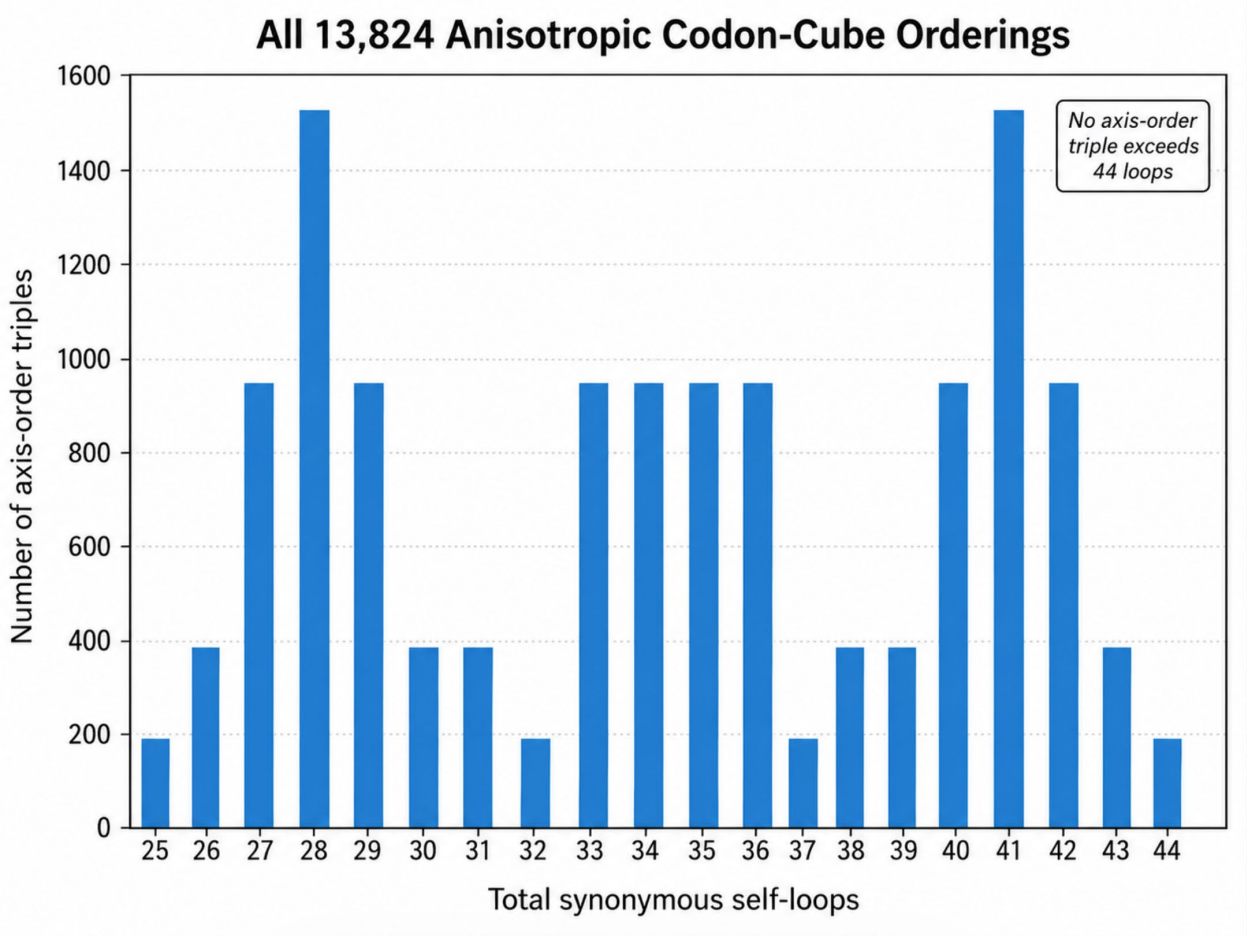

Figure 1. Distribution of synonymous self-loop counts across all 13,824 independently ordered codon cubes. The observed totals range from twenty-five to forty-four. No anisotropic axis-order triple exceeds the forty-four-loop value attained by the isotropic G-A-C-U and U-C-A-G cubes, while 192 oriented triples tie that maximum.

9.7 Reproducibility

The complete computational record consists of:

the Python source code,

the 13,824-row table of all axis-order triples,

the list of 192 oriented maximizing triples,

the reversal-reduced list of twenty-four undirected maxima,

the loop-count frequency distribution,

and

the summary of validation results.

Each output row contains the three nucleotide orders, the three axis-specific loop counts, and the total:

$\pi_1, \pi_2, \pi_3, L_1, L_2, L_3, L$.

This format permits any result to be checked without rerunning the complete search.

Independent reproduction requires only the standard RNA genetic-code table and the stated nearest-neighbor rule. No random numbers, fitted parameters, optimization heuristics, or numerical tolerances are involved.

The analytical proof and exhaustive computation converge on the same conclusion:

$L_{max} = 44$.

The computation additionally supplies the complete distribution and classification data needed to determine how the maximum is attained across the unrestricted anisotropic search space.

10. Classification of All Maximizing Axis Triples

10.1 Cartesian-Product Structure

The axis-separability theorem shows that a codon cube reaches the global maximum only when each axis independently reaches its own positional maximum.

The total loop count is

$L(\pi_1, \pi_2, \pi_3) = L_1(\pi_1) + L_2(\pi_2) + L_3(\pi_3)$.

The separate upper bounds are

$L_1 \leq 4$,

$L_2 \leq 1$,

and

$L_3 \leq 39$.

A cube attains forty-four loops only when all three equalities hold simultaneously:

$L_1(\pi_1) = 4$,

$L_2(\pi_2) = 1$,

and

$L_3(\pi_3) = 39$.

Let $\mathcal{P}_1$ denote the set of first-axis orders attaining four loops.

Let $\mathcal{P}_2$ denote the set of second-axis orders attaining one loop.

Let $\mathcal{P}_3$ denote the set of third-axis orders attaining thirty-nine loops.

The complete set of maximizing oriented axis triples is therefore

$\mathcal{P}\text{max} = \mathcal{P}_1 \times \mathcal{P}_2 \times \mathcal{P}_3$.

This Cartesian-product structure follows directly from axis independence. Any optimal first-axis order can be combined with any optimal second-axis order and any optimal third-axis order.

No additional compatibility condition is required because changing one axis order does not change the edge set or loop count on either of the other axes.

The maximizing family is therefore determined completely by three small position-specific order sets.

10.2 Oriented Count

The four oriented first-axis maximizing orders are

ACUG,

GACU,

GUCA,

and

UCAG.

Thus,

$|\mathcal{P}_1| = 4$.

The twelve oriented second-axis maximizing orders are

AGCU,

AGUC,

CAGU,

CGAU,

CUAG,

CUGA,

GACU,

GAUC,

UAGC,

UCAG,

UCGA,

and

UGAC.

Thus,

$|\mathcal{P}_2| = 12$.

The four oriented third-axis maximizing orders are

CUAG,

GACU,

GAUC,

and

UCAG.

Thus,

$|\mathcal{P}_3| = 4$.

The number of oriented maximizing triples is therefore

$|\mathcal{P}\text{max}| = |\mathcal{P}_1| \times |\mathcal{P}_2| \times |\mathcal{P}_3|$.

Substitution gives

$|\mathcal{P}\text{max}| = 4 \times 12 \times 4$.

Therefore,

$|\mathcal{P}\text{max}| = 192$.

This analytical count agrees exactly with the exhaustive search.

Every one of the 192 triples has the positional contribution vector

$(L_1, L_2, L_3) = (4, 1, 39)$.

Consequently, every member has total loop count

$L = 4 + 1 + 39 = 44$.

Conversely, every triple outside this Cartesian product fails to maximize at least one axis and therefore has fewer than forty-four loops.

The 192 cases represent

$192 / 13{,}824 = 1 / 72$

of the complete oriented search space.

Thus, approximately 1.39 percent of all independently ordered cubes attain the unrestricted maximum.

10.3 Reversal Reduction

An axis order and its complete reversal contain the same undirected adjacent nucleotide pairs.

For example,

G-A-C-U

and

U-C-A-G

both contain the undirected adjacencies

G-A,

A-C,

and

C-U.

They therefore generate the same undirected edge set on that axis.

The four first-axis maximizing orders form two reversal pairs:

ACUG ↔ GUCA,

and

GACU ↔ UCAG.

Thus, the first-axis maximizing set reduces from four oriented orders to two undirected path classes.

The twelve second-axis maximizing orders form six reversal pairs:

AGCU ↔ UCGA,

AGUC ↔ CUGA,

CAGU ↔ UGAC,

CGAU ↔ UAGC,

CUAG ↔ GAUC,

and

GACU ↔ UCAG.

Thus, the second-axis maximizing set reduces from twelve oriented orders to six undirected classes.

The four third-axis maximizing orders form two reversal pairs:

CUAG ↔ GAUC,

and

GACU ↔ UCAG.

Thus, the third-axis maximizing set reduces from four oriented orders to two undirected classes.

The reversal-reduced sizes are therefore

$|\mathcal{P}_1 / R| = 2$,

$|\mathcal{P}_2 / R| = 6$,

and

$|\mathcal{P}_3 / R| = 2$.

Here, R denotes identification of each order with its reverse.

Because reversal can be applied independently to all three axes, each undirected maximizing triple corresponds to

$2^3 = 8$

oriented representations.

This gives the same reduction directly:

192 / 8 = 24.

10.4 Twenty-Four Undirected Solutions

The number of reversal-reduced maximizing axis triples is

2 × 6 × 2.

Therefore,

24

distinct undirected anisotropic topologies attain the forty-four-loop maximum.

These twenty-four solutions are the Cartesian product of:

two optimal first-axis path classes,

six optimal second-axis path classes,

and

two optimal third-axis path classes.

The same proportion appears in the reversal-reduced search space:

24 / 1,728 = 1 / 72.

Thus, reversal reduction removes redundant orientations but does not change the relative frequency of maximum solutions.

The twenty-four undirected solutions are not twenty-four unrelated topologies discovered accidentally by brute force. They arise from a simple and exact combinatorial construction.

Each solution independently satisfies three positional requirements:

the first-axis path places C adjacent to both A and U;

the second-axis path places A adjacent to G;

and

the third-axis path preserves the required A-G and C-U pairs while also supporting two isoleucine connections.

Any combination of paths satisfying these three independent conditions reaches forty-four loops.

10.5 Isotropic Maxima Within the Family

An isotropic maximizing cube must use one nucleotide order that belongs simultaneously to all three oriented maximizing sets.

Therefore, its common order must lie in the intersection

$\mathcal{P}_1 \cap \mathcal{P}_2 \cap \mathcal{P}_3$.

The intersection contains exactly two oriented orders:

GACU

and

UCAG.

Thus, the only isotropic members of the 192-member maximizing family are

(GACU, GACU, GACU)

and

(UCAG, UCAG, UCAG).

These two cubes are complete reversals of one another and represent one undirected isotropic topology.

This explains the relationship between the earlier and present results.

Within the isotropic search, G-A-C-U is unique up to reversal.

Within the anisotropic search, the same maximum value is attained by many additional combinations because each axis can select its optimal path independently.

The isotropic G-A-C-U solution is special because one nucleotide order simultaneously satisfies the separate optimality conditions for all three codon positions.

Most anisotropic maxima use different optimal paths on different axes.

10.6 Figure 2: Structure of the Maximizing Family

Figure 2 should be placed here, after the oriented and reversal-reduced classifications have been established.

The figure may be presented as a three-column Cartesian-product diagram.

The first column should show the two reversal-reduced first-axis maximizing classes:

ACUG / GUCA,

and

GACU / UCAG.

The second column should show the six reversal-reduced second-axis maximizing classes:

AGCU / UCGA,

AGUC / CUGA,

CAGU / UGAC,

CGAU / UAGC,

CUAG / GAUC,

and

GACU / UCAG.

The third column should show the two reversal-reduced third-axis maximizing classes:

CUAG / GAUC,

and

GACU / UCAG.

A multiplication line beneath the columns should state

2 × 6 × 2 = 24 undirected maxima.

A second line may show the oriented count:

4 × 12 × 4 = 192 oriented maxima.

The isotropic G-A-C-U class should be visually identified as the unique path class appearing in all three columns.

Codon axis	Maximum loops	Oriented maximizing orders	Reversal-reduced classes
First position	4	4	2
Second position	1	12	6
Third position	39	4	2
Combined	44	192 triples	24 triples

Table 12. Cartesian-product classification of all codon-cube topologies attaining forty-four synonymous self-loops. Four oriented first-axis maximizers, twelve oriented second-axis maximizers, and four oriented third-axis maximizers combine independently to produce 192 oriented maximum triples. Identification of each axis order with its reverse reduces these to 2 × 6 × 2 = 24 distinct undirected solutions. G-A-C-U and U-C-A-G are the only isotropic oriented members of the maximizing family.

10.7 Meaning of Nonuniqueness

The existence of 192 oriented maxima does not weaken the statement that G-A-C-U is globally optimal.

Global optimality concerns the value attained:

L = 44.

Uniqueness concerns how many configurations attain that value.

The anisotropic search shows that G-A-C-U remains globally optimal because no cube exceeds forty-four. What changes is only the uniqueness classification.

The correct statements are therefore:

G-A-C-U and U-C-A-G are the unique isotropic maximizers.

No isotropic or anisotropic cube exceeds forty-four loops.

There are 192 oriented anisotropic axis triples attaining forty-four.

These reduce to twenty-four undirected topological solutions.

The additional maxima arise because anisotropy allows different codon positions to satisfy their optimality requirements through different nucleotide paths.

The isotropic result remains distinctive because G-A-C-U is one order capable of attaining all three positional maxima simultaneously.

The anisotropic classification therefore adds precision rather than contradiction. It separates two mathematical properties that were combined in the isotropic result:

maximum loop value,

and

uniqueness of the nucleotide ordering.

The maximum value survives the unrestricted search unchanged.

The uniqueness does not.

This distinction is one of the central conclusions of the pape

11. Symmetry, Reproducibility, and Extensions

11.1 Axis-Reversal Symmetry

A nucleotide order and its complete reverse generate the same undirected path.

For example,

G-A-C-U

and

U-C-A-G

contain the same three undirected nucleotide adjacencies:

G-A,

A-C,

and

C-U.

Only the direction in which the path is written changes.

Let $R\pi$ denote the reversal of nucleotide order π. If

$\pi = (s_0, s_1, s_2, s_3)$,

then

$R\pi = (s_3, s_2, s_1, s_0)$.

The corresponding undirected axis edge sets are identical:

$E_p(\pi) = E_p(R\pi)$.

Therefore, the synonymous contribution is also unchanged:

$L_p(\pi) = L_p(R\pi)$.

This symmetry applies independently to the three codon axes. Reversing the first-axis order does not require reversal of either the second or third axis.

Thus, the oriented triple

(π_1, π_2, π_3)

belongs to the same undirected topological class as

$(R\pi_1, \pi_2, \pi_3)$,

$(\pi_1, R\pi_2, \pi_3)$,

$(\pi_1, \pi_2, R\pi_3)$,

and the four additional combinations formed by reversing two or all three axes.

Each undirected triple therefore has

$2^3 = 8$

oriented representations.

This explains the reduction

13,824 / 8 = 1,728

from oriented axis triples to reversal-reduced undirected classes.

The same symmetry reduces the 192 oriented maxima to

192 / 8 = 24

undirected maxima.

Axis reversal does not alter the loop statistic because the graph is undirected. If edge orientation were introduced in a future model, reversal could become significant. In the present analysis, however, G-A-C-U and U-C-A-G are topologically identical.

11.2 Independent Computational Verification

The exhaustive result can be verified by several independent computational strategies.

The first method constructs the full 4 × 4 × 4 coordinate lattice for every axis-order triple. It generates nearest-neighbor edges by incrementing one coordinate at a time and counts synonymous endpoint labels.

The second method does not require explicit three-dimensional coordinates. For each axis, it identifies the three adjacent nucleotide pairs in the selected order. It then generates all codon pairs differing by one of those nucleotide pairs at the corresponding codon position.

The third method uses the axis-separable formulas directly. It calculates L_1 for each of the twenty-four first-axis orders, L_2 for each second-axis order, and L_3 for each third-axis order. The complete 13,824-row result table is then generated by adding all possible combinations of the three one-axis results.

All three methods should produce identical totals.

Several fixed assertions provide strong error checks.

The genetic-code mapping must contain sixty-four codons.

Each cube must contain forty-eight edges per axis.

The total edge count must equal 144.

The search must evaluate exactly 13,824 oriented triples.

Every axis order must have the same loop contribution as its reverse.

The isotropic G-A-C-U case must produce

$(L_1, L_2, L_3) = (4, 1, 39)$.

The maximum must equal forty-four.

The number of oriented maxima must equal 192.

The number of reversal-reduced maxima must equal twenty-four.

The full loop-count frequency table must sum to 13,824.

Because the calculation contains no random sampling, floating-point approximation, numerical optimization, or fitted parameters, exact agreement should be expected across independent implementations.

A discrepancy would indicate a difference in the genetic-code table, the treatment of stop X, the definition of nearest-neighbor adjacency, or the handling of duplicate edges.

11.3 Position-Specific Topology

The positional maxima

4, 1, and 39

show that the three codon positions have radically different synonymous graph structures.

The first position contributes only a small number of bridges between separated codon blocks. These involve leucine and arginine and depend on the nucleotide adjacencies C-U and C-A.

The second position contributes only one synonymous edge, and that edge connects two stop codons. No amino-acid pair differing only at the second position remains synonymous.

The third position contributes thirty-nine loops because most genetic-code degeneracy is organized through the final nucleotide.

The ratio of the axis maxima is

4 : 1 : 39.

The third-position maximum accounts for

39 / 44

of the unrestricted total.

Numerically, this is approximately

0.886.

Thus, about 88.6 percent of the maximum loop count is contributed by third-position edges.

This asymmetry is not imposed by the cube geometry. Each axis contains the same number of vertices and the same forty-eight edges.

The asymmetry is produced by the standard genetic-code assignments.

If amino-acid labels were randomly reassigned to codons, the three positions would generally show different and less structured synonymous counts. The observed values therefore describe how degeneracy is distributed across the actual code.

The anisotropic model makes this positional structure explicit. Instead of treating the cube as one indivisible object, it reveals three independent topological layers.

11.4 Alternative Graph Statistics

The synonymous self-loop count measures exact preservation of translated identity. It does not measure every potentially important feature of codon topology.

One extension is the number of connected components formed by the codons encoding each amino acid.

A synonymous family may contain several edges but still be divided into disconnected blocks. Leucine and arginine illustrate this distinction because each occupies a four-codon block and a two-codon branch.

Another statistic is the mean shortest-path distance among synonymous codons.

For amino acid r, let $\mathcal{C}_r$ be its codon set. The average pairwise distance can be written as

$\bar{d}_r$ = average of d(c,d) over all distinct c and d in $\mathcal{C}_r$.

A smaller value indicates greater global compactness even when codons are not directly adjacent.

A connector-length measure could calculate the minimum number of cube edges required to join all codons encoding one amino acid.

The multigraph may also be analyzed through edge multiplicities. Some amino-acid pairs are connected by several codon-level edges, while others are connected only once.

Other possible statistics include:

the number of synonymous connected components,

the diameter of each synonymous codon set,

the total length of a minimum connecting tree,

the clustering of chemically similar amino acids,

and

the distribution of nonsynonymous edge multiplicities.

These measures may identify optimal axis orders different from those selected by self-loop count alone.

11.5 Weighted Topology

The present loop statistic assigns weight one to every synonymous edge and weight zero to every nonsynonymous edge.

A weighted model can preserve more biological information.

One possibility is to distinguish transitions from transversions. A nucleotide substitution between two purines or between two pyrimidines could receive a different weight from a purine-pyrimidine substitution.

Another possibility is to weight the three codon positions differently. Errors at the first, second, and third positions do not have equal consequences for translation.

The weighted score could be written as

$W = w_1L_1 + w_2L_2 + w_3L_3$,

where w_1, w_2, and w_3 are position-specific weights.

Codon-usage frequencies could also be incorporated. An edge involving common codons may contribute more strongly to an organism-specific score than an edge involving rare codons.

A physicochemical model could assign a cost to nonsynonymous edges rather than treating them all equally.

For an edge joining codons c and d, define a property difference

$\Delta(c,d)$.

This difference could measure hydropathy, residue volume, charge, or a multidimensional amino-acid distance.

A total edge cost could then be written as

$C = \Sigma\ \Delta(c,d)$,

with the sum taken over all 144 cube edges.

A favorable nucleotide ordering might minimize C even if it does not maximize the exact synonymous loop count.

These weighted extensions should be treated as new optimization problems. Their results should not be assumed from the forty-four-loop theorem.

11.6 Limits of the Result

The forty-four-loop bound is exact for the graph defined in this paper.

The graph uses the standard RNA genetic code.

Each codon position contains a linear path through four nucleotide states.

Only consecutive nucleotide states are treated as nearest neighbors.

The three axis paths may be chosen independently.

Edges are undirected.

Stop X is included as one translated label.

Every synonymous edge has weight one.

Every nonsynonymous edge has weight zero.

Under these definitions,

Lmax = 44.

Changing any of these assumptions creates a different mathematical problem.

If stop X is excluded, the second-axis maximum becomes zero and the total bound changes.

If every one-nucleotide substitution is included, the graph becomes the complete Hamming-distance-one graph rather than the 144-edge ordered cube.

If nucleotide paths are replaced by cycles, each axis gains an additional adjacency.

If edges are directed, reversal symmetry may no longer hold.

If physicochemical similarity is weighted, a nonsynonymous edge may contribute partially rather than zero.

If codon usage is organism specific, the score may differ among species.

The present theorem therefore should not be described as universal optimality under every conceivable genetic-code metric.

Its precise conclusion is:

Among all 13,824 independently ordered linear codon cubes, the maximum number of unweighted nearest-neighbor edges preserving amino-acid or stop identity is forty-four.

This result is analytically proven, computationally exhaustive, and fully reproducible under the stated definitions.

12. Synthesis and Conclusions

12.1 Answer to the Anisotropic Question

The central question of this paper was whether allowing the first, second, and third codon axes to use independent nucleotide orders could produce more synonymous nearest-neighbor edges than the previously established isotropic G-A-C-U maximum.

The unrestricted search allowed separate nucleotide orders

π_1, π_2, and π_3

for codon positions one, two, and three.

Because each axis admits twenty-four oriented nucleotide orders, the complete search space contained

$24^3 = 13{,}824$

axis-order triples.

The answer is definitive:

No anisotropic codon cube exceeds forty-four synonymous self-loops.

The unrestricted maximum is

$L_{max} = 44$.

This result was established in two independent ways.

First, the total loop count was decomposed analytically into three independent axis contributions.

Second, every one of the 13,824 oriented axis-order triples was evaluated computationally.

The analytical proof and exhaustive search agreed exactly.

The anisotropic extension therefore strengthens the earlier isotropic result. Forty-four is not merely the largest value obtained when one common nucleotide order is imposed on all three codon positions. It is the global maximum across all independently ordered linear codon cubes.

12.2 Exact Mathematical Reason

The decisive mathematical property is axis separability.

Every nearest-neighbor edge changes exactly one codon position. The 144 cube edges therefore divide into three disjoint sets:

48 first-position edges,

48 second-position edges,

and

48 third-position edges.

The total synonymous loop count separates as

$L(\pi_1, \pi_2, \pi_3) = L_1(\pi_1) + L_2(\pi_2) + L_3(\pi_3)$.

Each term depends only on the nucleotide order assigned to its own codon axis.

The first-axis order cannot change the second- or third-axis loop counts.

The second-axis order cannot change the first- or third-axis counts.

The third-axis order cannot change the first- or second-axis counts.

The optimization problem therefore separates into three independent one-axis problems.

Their exact maxima are

$\max L_1 = 4$,

$\max L_2 = 1$,

and

$\max L_3 = 39$.

The unrestricted maximum is consequently

$L_{max} = 4 + 1 + 39$.

Therefore,

$L_{max} = 44$.

This is an exact bound rather than an empirical estimate. No interaction among the three axis orders can produce an additional loop because the edge sets are disjoint and the total statistic is additive.

12.3 Codon-Position Asymmetry

The separate maxima reveal a striking asymmetry among the three codon positions:

4 : 1 : 39.

The first position supports four possible synonymous bridges. Two connect the separated leucine codon blocks, and two connect the separated arginine blocks.

The second position supports only one synonymous edge. This edge joins the stop codons UAA and UGA and requires A and G to be adjacent in the second-axis nucleotide path.

The third position supports thirty-nine synonymous edges. These arise from the extensive organization of genetic-code degeneracy through the final nucleotide.

The third-axis contribution includes:

twenty-four invariant edges from eight complete fourfold-degenerate codon blocks,

nine edges from ordinary twofold-degenerate amino-acid families,

two isoleucine edges,

three additional edges from the two-codon branches of leucine, arginine, and serine,

and

one stop-codon edge.

Thus, the third position contributes

$39 / 44 \approx 0.886$

of the maximum total.

Approximately 88.6 percent of the unrestricted maximum therefore comes from third-position topology.

This positional imbalance is not produced by the geometry of the cube. Each axis contains the same number of coordinate states and the same forty-eight edges.

The asymmetry is produced by the standard genetic-code assignments.

12.4 Relationship to the Isotropic Result

The earlier isotropic analysis showed that G-A-C-U and its reverse U-C-A-G uniquely maximize synonymous loops when one nucleotide order is applied identically to all three codon axes.

The G-A-C-U cube has the contribution vector

$(L_1, L_2, L_3) = (4, 1, 39)$.

It therefore reaches the independent maximum on every codon axis simultaneously.

This explains why G-A-C-U remains globally optimal after the isotropic restriction is removed.

The anisotropic search does not produce a higher value because no axis can exceed the contribution already attained by G-A-C-U.

What changes is uniqueness.

Within the isotropic search, only

(GACU, GACU, GACU)

and

(UCAG, UCAG, UCAG)

attain forty-four.

These two oriented cubes are complete reversals and represent one undirected isotropic topology.

Within the unrestricted anisotropic search, many additional combinations attain the same maximum by selecting different optimal orders for the three positions.

The correct interpretation is therefore:

G-A-C-U remains globally optimal in value.

G-A-C-U is unique up to reversal only within the isotropic search.

Anisotropy creates additional ties but no improvement.

12.5 Complete Classification of the Maxima

The maximizing anisotropic family has an exact Cartesian-product structure.

There are four oriented first-axis orders attaining four loops.

There are twelve oriented second-axis orders attaining one loop.

There are four oriented third-axis orders attaining thirty-nine loops.

Any combination of these independently optimal orders produces forty-four total loops.

The number of oriented maximizing triples is therefore

$4 \times 12 \times 4 = 192$.

The exhaustive computation found exactly 192 cases with forty-four loops, confirming the analytical classification.

Each nucleotide path is equivalent to its complete reversal in the undirected cube.

The four first-axis maximizers reduce to two reversal classes.

The twelve second-axis maximizers reduce to six reversal classes.

The four third-axis maximizers reduce to two reversal classes.

The number of undirected maximizing triples is therefore

$2 \times 6 \times 2 = 24$.

Equivalently,

$192 / 8 = 24$,

because each undirected triple has eight independently reversed oriented representations.

The complete anisotropic classification is therefore:

13,824 oriented axis-order triples examined,

192 oriented maxima,

1,728 reversal-reduced undirected triples,

and

24 reversal-reduced maxima.

The maximizing oriented triples occupy

$192 / 13{,}824 = 1 / 72$

of the unrestricted search space.

Thus, approximately 1.39 percent of all independently ordered cubes attain the global bound.

12.6 Final Conclusion

The unrestricted topology of the ordered genetic-code cube is governed by an exact and simple principle.

The total number of synonymous nearest-neighbor edges separates into independent contributions from the first, second, and third codon positions.

These contributions have exact maxima of four, one, and thirty-nine.

Their sum fixes the global bound:

$L_{max} = 44$.

Exhaustive enumeration of all 13,824 independently ordered codon cubes confirms that

0 cases exceed 44,

while

192 oriented cases attain 44.

After reversal symmetry is removed, these maxima correspond to twenty-four distinct undirected axis-order triples.

The earlier G-A-C-U result therefore survives the unrestricted test in its most important sense. G-A-C-U and U-C-A-G attain the largest possible number of synonymous nearest-neighbor edges even when the three codon axes are allowed to vary independently.

The enlarged search changes only the uniqueness statement.

The final result is:

G-A-C-U is the unique isotropic maximum up to reversal.

No anisotropic ordering exceeds its value of forty-four.

Multiple anisotropic axis-order triples tie, but none improves upon, the G-A-C-U maximum.

The forty-four-loop bound is therefore both analytically proven and computationally exhaustive under the stated graph definition.

13. References and Supplementary Computational Records

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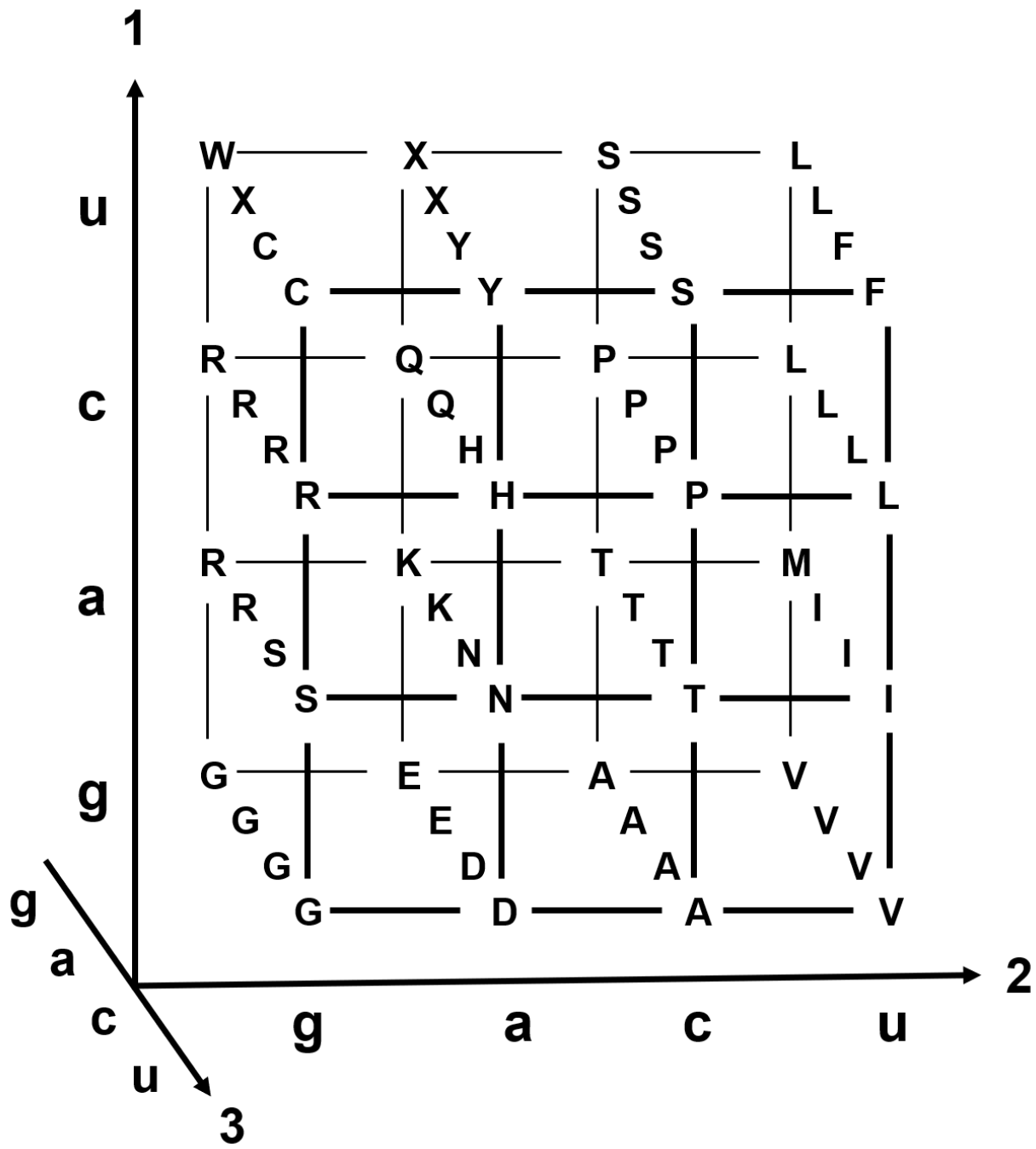
The author has published over 5,500 AI-assisted papers at: https://www.researchgate.net/profile/Douglas-Youvan/research . Google Scholar has indexed these preprints, and if you want a particular reference, the AI mode of a Google search (Gemini) has efficiently catalogued these preprints.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